APPENDIX

References of Interest

1. Abstract by Sukharev S.I., Klenchin V.A., Serov S.M., Chernomordik L.V. and Chizmadzhev YuA, *Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores*, Biophys J. 1992 Nov; 63(5):1320-7.

   Copy enclosed.

2. U.S. Patent No. 5,371,003 (Murray *et al.*), filed on September 23, 1993 and entitled "Electrotransformation Process".

3. Jacobs, DF and Timmer, VR (2005). *Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function.* New Forests 30:147-166.

   http://www.agriculture.purdue.edu/fnr/HTIRC/documents/JacobsandTimmer2005_000.pdf

1: Biophys J. 1992 Nov;63(5):1320-7. Related Articles, Links

Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores.

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It has been shown recently that electrically induced DNA transfer into cells is a fast vectorial process with the same direction as DNA electrophoresis in an external electric field (Klenchin, V. A., S. I. Sukharev, S. M. Serov, L. V. Chernomordik, and Y. A. Chizmadzhev. 1991. Biophys. J. 60:804-811). Here we describe the effect of DNA interaction with membrane electropores and provide additional evidences for the key role of DNA electrophoresis in cell electrotransfection. The assay of electrically induced uptake of fluorescent dextrans (FDs) by cells shows that the presence of DNA in the medium during electroporation leads to a sharp increase in membrane permeability to FDs of M(r) < 20,000. The permeability increases with DNA concentration and the effect is seen even if FD is added to the cell suspension a few minutes after pulse application. The longer the DNA fragment, the greater the increase in permeability. The use of a two-pulse technique allows us to separate two effects provided by a pulsed electric field: membrane electroporation and DNA electrophoresis. The first pulse (6 kV/cm, 10 microseconds) creates pores efficiently, whereas transfection efficiency (TE) is low. The second pulse of much lower amplitude, but substantially longer (0.2 kV/cm, 10 ms), does not cause poration and transfection by itself but enhances TE by about one order of magnitude. In two-pulse experiments, TE rises monotonously with the increase of the second pulse duration. By varying the delay duration between the two pulses, we estimate the lifetime of electropores (which are DNA-permeable in conditions of low electric field) as tens of seconds.(ABSTRACT TRUNCATED AT 250 WORDS)

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